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NEB expressions

a scientific update

IN THIS ISSUE

NEW ENGLAND BioLabs®

Minding your caps and tails – considerations for functional mRNA synthesis

by Breton Hornblower, Ph.D., G. Brett Robb, Ph.D. and George Tzertzinis, Ph.D., New England Biolabs, Inc.

Applications of synthetic mRNA have grown and become considerably diversified in recent years. Examples include the generation of pluripotent stem cells (1-3), vaccines and therapeutics (4-6), and CRISPR/Cas9 genome editing applications (7-9). The basic requirements for a functional mRNA – a 7-methylguanylate cap at the 5´ end and a poly(A) tail at the 3´ end – must be added in order to obtain efficient translation in eukaryotic cells. Additional considerations can include the incorporation of internal modified bases, modified cap structures and polyadenylation strategies. Strategies for *in vitro* synthesis of mRNA vary according to the desired scale of synthesis.

To read the full article, please visit **www.neb.com/minding-your-caps-and-tails**

A nascent mRNA, synthesized in the nucleus, undergoes modifications before it can be translated into proteins in the cytoplasm. For a mRNA to be functional, it requires modified 5´ and 3´ ends and a coding region (i.e., an open reading frame (ORF) encoding the protein of interest) flanked by the untranslated regions (UTRs). The nascent mRNA (pre-mRNA) undergoes two significant modifications in addition to splicing. During synthesis, a 7-methylguanylate structure, also known as a "cap", is added to the 5´ end of the pre-mRNA, via 5´ → 5´ triphosphate linkage. This cap protects the mature mRNA from degradation, and also serves a role in nuclear export and efficient translation.

The second modification occurs post-transcriptionally at the 3´ end of the nascent RNA molecule, and is characterized by addition of approximately 200 adenylate nucleotides (poly(A) tail). The addition of the poly(A) tail confers stability to the mRNA, aids in the export of the mRNA to the cytosol, and is involved in the formation of a translation-competent ribonucleoprotein (RNP), together with the 5´ cap structure. The mature mRNA forms a circular structure (closed-loop) by bridging the cap to the poly(A) tail via the cap-binding protein eIF4E (eukaryotic initiation factor 4E) and the poly(A)-binding protein, both of which interact with eIF4G (eukaryotic initiation factor 4G) (10).

RNA can be efficiently synthesized *in vitro* (by *in vitro* transcription, IVT) with prokaryotic phage polymerases, such as T7, T3 and SP6. The cap and poly(A) tail structures characteristic of mature mRNA can be added during or after the synthesis by enzymatic reactions with capping enzymes and Poly(A) Polymerase (NEB #M0276), respectively.

There are several factors to consider when planning for IVT-mRNA synthesis that will influence the ease-of-experimental setup and yield of the final mRNA product.

In vitro transcription

There are two options for the *in vitro* transcription (IVT) reaction depending on the capping strategy chosen: standard synthesis with enzyme-based capping following the transcription reaction (post-transcriptional capping) or incorporation of a cap analog during transcription (co-transcriptional capping) (Figure 1). Method selection will depend on the scale of mRNA synthesis required and number of templates to be transcribed.

Transcription for enzyme-based capping (post-transcriptional capping)

Standard RNA synthesis reactions produce the highest yield of RNA transcript (typically ≥100 µg per 20 µl in a 1 hr reaction using the HiScribe Quick T7 High Yield RNA Synthesis Kit, NEB #E2050S). Transcription reactions are highly scalable.

Following transcription, the RNA is treated with DNase I (NEB #M0303) to remove the DNA template, and purified using an appropriate column, kit or magnetic beads, prior to capping. This method produces high yields of RNA with 5´-triphosphate termini that must be converted to cap structures. In the absence of template-encoded poly(A) tails, transcripts produced using this method bear 3´ termini that also must be polyadenylated in a separate enzymatic step, as described below in "Post-transcriptional capping and Cap-1 methylation".

Transcription with dinucleotide co-transcriptional capping

In co-transcriptional capping, a cap analog is introduced into the transcription reaction, along with the four standard nucleotide triphosphates, in an optimized ratio of cap analog to GTP 4:1. This allows initiation of the transcript with the cap structure in a large proportion of the synthesized RNA molecules. This approach produces a mixture of transcripts, of which ~80% are capped, and the remainder have 5´-triphosphate ends. Decreased overall yield of RNA products results from the lower concentration of GTP in the reaction.

There are several cap analogs used in co-transcriptional RNA capping (3). The most common are the standard 7-methyl guanosine (m7G) cap analog and anti-reverse cap analog (ARCA), also known as 3´ O-me 7-meGpppG cap analog. ARCA is methylated at the 3´ position of the m7G, preventing RNA elongation by phosphodiester bond formation at this position. Thus, transcripts synthesized using ARCA contain 5´-m7G cap structures in the correct orientation, with the 7-methylated G as the terminal residue. In contrast, the m7G cap analog can be incorporated in either the correct or the reverse orientation.

HiScribe T7 ARCA mRNA Synthesis kits (NEB #E2060 and #E2065) contain reagents, including an optimized mix of ARCA and NTPs, for streamlined reaction setup for synthesis of co-transcriptionally capped RNAs.

Transcription with CleanCap Reagent AG co-transcriptional capping

The use of CleanCap reagent AG results in significant advantages over traditional dinucleotide co-transcriptional capping. CleanCap Reagent AG is a trinucleotide with a 5´-m7G joined by a 5´ → 5´ triphosphate linkage to an AG sequence. The adenine has a methyl group on the 2´-O position. The incorporation of this trinucleotide in the beginning of a transcript results in a Cap-1 structure.

In order to use CleanCap Reagent AG in an *in vitro* transcription reaction the template must contain an AG in place of a GG following the T7 promoter in the initiation sequence.

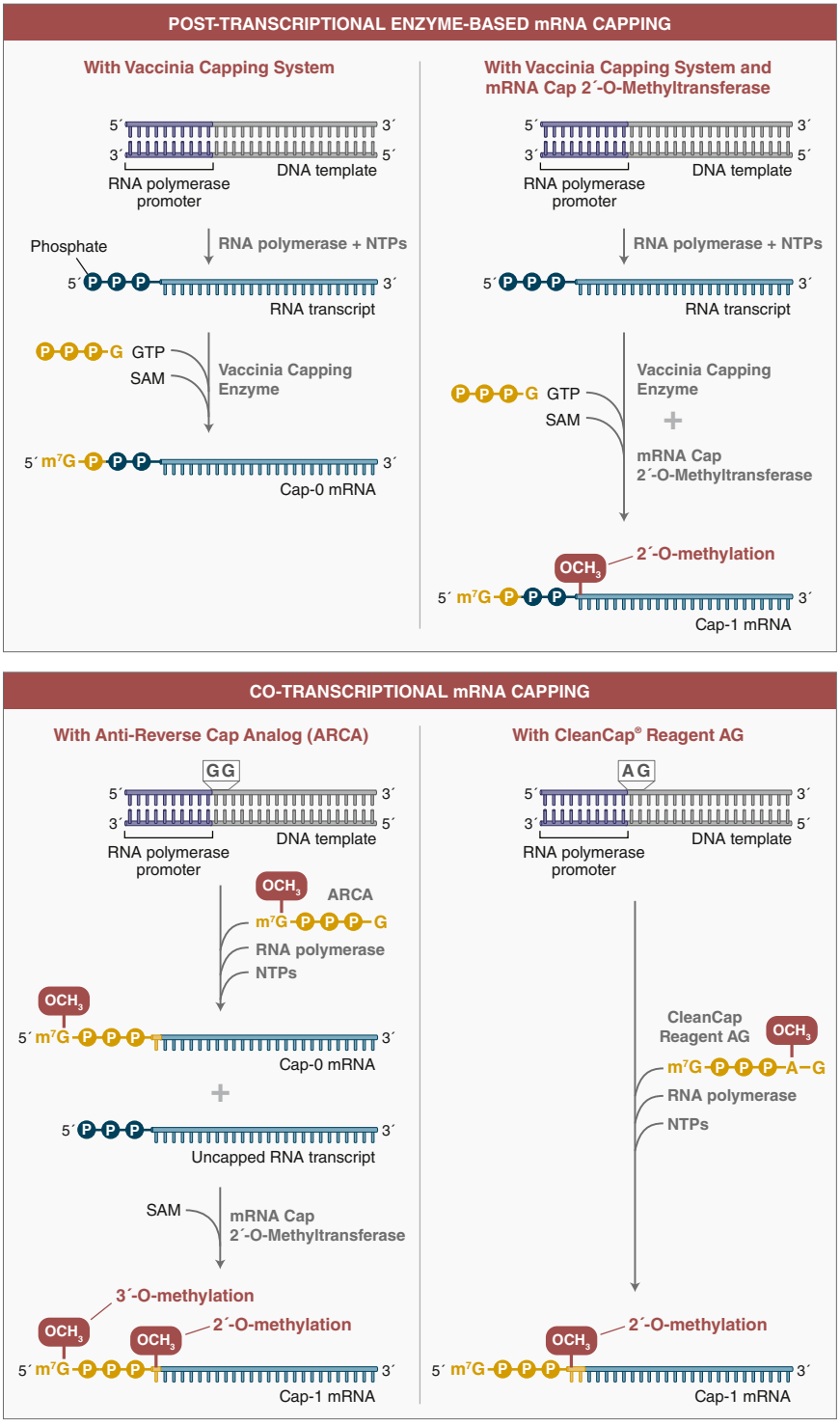
Unlike traditional co-transcriptional capping, reduction of GTP concentration is not required and therefore yield is higher and high capping effiencies, >95%, are achieved.

Transcription with complete substitution with modified nucleotides

RNA synthesis can be carried out with a mixture of modified nucleotides in place of the regular mixture of A, G, C and U triphosphates. For expression applications, the modified nucleotides of choice are the naturally occurring 5´-methylcytidine and/or pseudouridine in the place of C and U, respectively. These have been demonstrated to confer desirable properties to the mRNA, such as increased mRNA stability, increased translation, and reduced immune response in the key applications of protein replacement and stem-cell differentiation (1). It is important to note that nucleotide choice can influence the overall yield of mRNA synthesis reactions.

Fully substituted RNA synthesis can be achieved using the HiScribe T7 mRNA Kit with CleanCap Reagent AG (NEB #E2080), HiScribe T7 High-Yield RNA Synthesis Kit (NEB #E2040) or HiScribe SP6 RNA Synthesis Kit (NEB #E2070) in conjunction with NTPs with the desired modification. Transcripts made with complete replacement of one or more nucleotides may be post-transcriptionally capped (see next section), or may be co-transcriptionally capped by including

Figure 1: ***In vitro*** **transcription options based upon capping strategy**



Enzyme-based capping (top) is performed after in vitro transcription using 5´-triphosphate RNA, GTP, and S-adenosyl- methionine (SAM). Cap-0 mRNA can be converted to Cap-1 mRNA using mRNA cap 2´-O-methyltransferase (MTase) and SAM in a subsequent or concurrent reaction. The methyl group transferred by the MTase to the 2´-O of the first nucleotide of the transcript is indicated in red. Conversion of ~100% of 5´-triphosphorylated transcripts to capped mRNA is routinely achievable using enzyme-based capping.

Co-transcriptional capping (bottom) uses an mRNA cap analog, shown in yellow, in the transcription reaction. For ARCA (anti-reverse cap analog) (left),the cap analog is incorporated as the first nucleotide of the transcript. ARCA contains an additional 3´-O-methyl group on the 7-methylguanosine to ensure incorporation in the correct orientation. The 3´-O-methyl modification does not occur in natural mRNA caps. Compared to reactions not containing cap analog, transcription yields are lower. ARCA- capped mRNA can be converted to cap 1 mRNA using mRNA cap 2´-O-MTase and SAM in a subsequent reaction. CleanCap Reagent AG (right) uses a trinucleotide cap analog that requires a modified template initiation sequence. A natural Cap-1 structure is accomplished in a co-transcriptional reaction.

"GMP-grade" is a branding term NEB uses to describe reagents manufactured at NEB's Rowley facility. The Rowley facility was designed to manufacture reagents under more rigorous infrastructure and process controls to achieve more stringent product specifications and customer requirements. Reagents manufactured at NEB's Rowley facility are manufactured in compliance with ISO 9001 and ISO 13485 quality management system standards. However, at this time, NEB does not manufacture or sell products known as Active Pharmaceutical Ingredients (APIs), nor does NEB manufacture its products in compliance with all of the Current Good Manufacturing Practice regulations.

CleanCap Reagent AG, ARCA or another cap analog, as described previously.

If partial replacement of nucleotides is desired, the HiScribe T7 ARCA mRNA Synthesis Kits (NEB #E2060 and #E2065), may be used with added modified NTPs, to produce co-transcriptionally capped mRNAs, as described above. Alternatively, the HiScribe T7 Quick RNA Synthesis Kit (NEB #E2050) may be used to prepare transcripts for post-transcriptional capping.

Post-transcriptional capping and Cap-1 methylation

Post-transcriptional capping is often performed using the mRNA capping system from *Vaccinia* virus. This enzyme complex converts the 5´-triphosphate ends of *in vitro* transcripts to m7G-cap (Cap-0) required for efficient protein translation in eukaryotes. The *Vaccinia* Capping System (NEB #M2080) comprises three enzymatic activities (RNA triphosphatase, guanylyltransferase, guanine N7-methyltransferase) that are necessary for the formation of the complete Cap-0 structure, m7Gppp5´N, using GTP and the methyl donor S-adenosylmethionine (SAM). As an added option, the inclusion of the mRNA Cap 2´ O-Methyltransferase (NEB #M0366) in the same reaction results in formation of the Cap-1 structure (m7Gppp5´Nm), a natural modification in many eukaryotic mRNAs responsible for evading cellular innate immune response against foreign RNA. This enzyme-based capping approach results in a high proportion of capped message, and it is easily scalable. The resulting capped RNA can be further modified by poly(A) addition before final purification.

A-tailing using *E. coli* Poly(A) Polymerase

The poly(A) tail confers stability to the mRNA and enhances translation efficiency. The poly(A) tail can be encoded in the DNA template by using an appropriately tailed PCR primer, or it can be added to the RNA by enzymatic treatment with *E. coli* Poly(A) Polymerase (NEB #M0276). The length of the added tail can be adjusted by titrating the Poly(A) Polymerase in the reaction.

For mRNA synthesis from templates with encoded poly(A) tails, the HiScribe T7 ARCA mRNA Synthesis Kit (NEB #E2065) provides an optimized formulation for co-transcriptionally capped transcripts.

In summary, when choosing the right workflow for your functional mRNA synthesis needs, you must balance your experimental requirements for the mRNA (e.g., internal modifed nucleotides) with scalability (i.e., ease-of-reaction setup vs. yield of final product).

Products from NEB are available for each step of the RNA synthesis workflow. GMP-grade* reagents suitable for the large scale manufacture of therapeutics mRNA are available through our Customized Solution Group.

References:

1. *Warren, L., et al. (2010) Cell Stem Cell, 7, 618-630.*
2. *Angel, M. and Yanik, M.F. (2010) PLoS One, 5:e11756.*
3. *Yakubov, E., et al. (2010) Biochem. Biophys. Res. Commun. 394, 189.*
4. *Haas, E.J., et al. (2021) Lancet, 397, 1819-1829.*
5. *Thompson, M.G., et al. (2021) N. Engl. J. Med. 384, 403-416.*
6. *Ramaswamy, S., et al. (2017) Proc. Natl. Acad. Sci. USA, 114, E1941-E1950.*
7. *Ma, Y., et al. (2014) PLoS One, 9:e89413.*
8. *Ota, S., et al. (2014) Genes Cells, 19, 555-564.*
9. *Bassett, A. R., et al. (2013) Cell Rep. 4, 220–228.*
10. *Wells, S.E., et al. (1998) Molecular Cell 2, 135–140.*

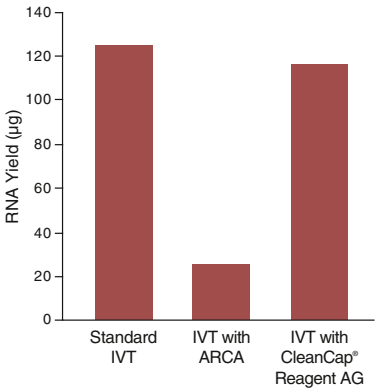
New tools for your (m)RNA Synthesis (IVT)

New Products:

HiScribe™ T7 mRNA Kit with CleanCap® Reagent AG

The HiScribe T7 mRNA Kit with CleanCap Reagent AG utilizes an optimized RNA synthesis formulation and trinucleotide cap analog technology to co-transcriptionally cap mRNAs containing a natural Cap-1 structure in a single simplified reaction without compromising RNA yield. Using a DNA template with a T7 promoter sequence followed by an AG initiation sequence and an encoded poly(A) tail, mRNAs can be transcribed with a 5´-m7G Cap-1 structure that is polyadenylated, translationally competent and able to evade the cellular innate immune response.

Comparison of RNA Yields from in vitro Reagent AG Transcription Reactions with no cap analog, ARCA, or CleanCap Reagent AG



All reactions were performed with 5 mM CTP, 5 mM UTP and 6 mM ATP. Standard IVT reactions included 5 mM GTP and no cap analog. ARCA reactions contained a 4:1 ratio of ARCA:GTP (4mM:1mM). IVT with CleanCap Reagent AG contained 5 mM GTP and 4 mM CleanCap Reagent AG and was performed as described (Standard mRNA Synthesis, HiScribe T7 mRNA Kit with CleanCap Reagent AG). Reactions were incubated for 2 hours at 37°C, purified and quantified by NanoDrop.

Advantages:

- Synthesize and cap mRNA in a single reaction
- Evade immune response with natural Cap-1 structure
- Generate high yields of mRNA, up to 1.8 mg per kit
- Suitable for full or partial modified nucleotide substitutions

To learn more, please visit
www.neb.com/E2080

Ordering information:

Product	NEB #	Size
HiScribe T7 mRNA Kit with CleanCap Reagent AG	E2080S	20 rxns

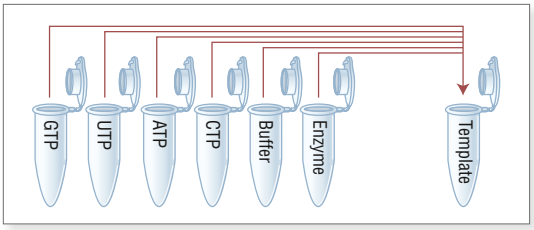
Also available:

HiScribe™ T7 (Quick) High Yield RNA Synthesis Kit

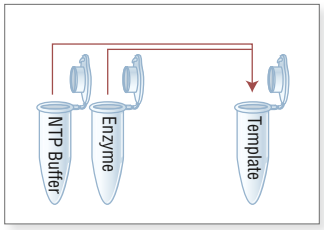
The HiScribe T7 High Yield RNA Synthesis Kit (NEB #E2040) delivers robust high yield RNA synthesis (up to 180µg/reaction) for a wide range of template sizes. Flexible protocols ensure that performance is maintained even under demanding conditions, such as extended reaction time using very low amounts of template. Protocols are included for partial or complete incorporation of modified or labeled nucleotides in the transcript body, and cap analogs at the RNA 5' end.

The HiScribe T7 Quick High Yield RNA Synthesis Kit (NEB #E2050) utilizes a master mix format, allowing for faster reaction setup. DNase I and lithium chloride are included for DNA template removal and quick RNA purification.

HiScribe T7 High Yield RNA Synthesis Kit



HiScribe T7 Quick High Yield RNA Synthesis Kit



Advantages:

- Streamlined format & Quick Workflows
- High Yield – up to 180 µg of RNA from a standard 20 µl reaction
- Flexibility – enables incorporation of cap analogs, radiolabeled and modified nucleotides

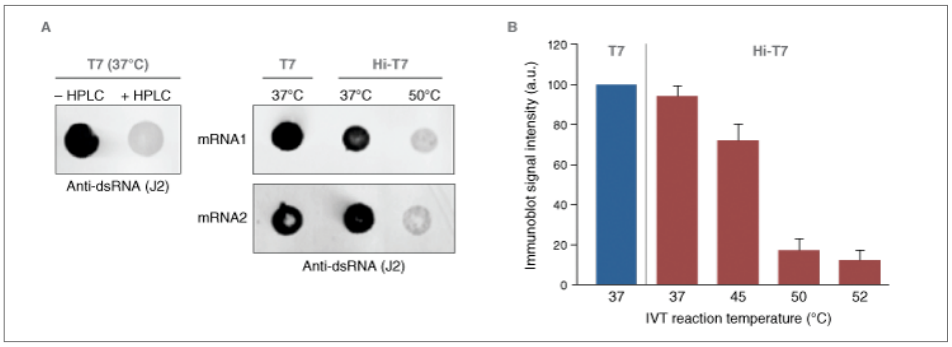
To learn more, please visit
www.neb.com/E2040

Ordering information:

Product	NEB #	Size
HiScribe T7 High Yield RNA Synthesis Kit	E2040S	50 rxns
HiScribe T7 Quick High Yield RNA Synthesis Kit	E2050S	50 rxns
HiScribe SP6 RNA Synthesis Kit	E2070S	50 rxns

Hi-T7® RNA Polymerase for reduced dsRNA by-product formation

Hi-T7 RNA Polymerase is an engineered DNA-dependent RNA polymerase that is highly specific for T7 phage promoters, designed for *in vitro* transcription of RNA at higher temperatures and recommended for experienced users interested in building and optimizing their own *in vitro* transcription reactions.



Immunoblot using an anti-dsRNA antibody (J2) shows presence of dsRNA by-products in the IVT reactions for both T7 and Hi-T7 RNA Polymerases when IVT is performed at 37°c. HPLC purification of the IVT RNA eliminates dsRNA by-products. dsRNA by-products are reduced when IVT is performed at 50°c (or higher temperatures) with Hi-T7.

Advantages:

- Active from 37-56°C, optimal incubation temperature is 50-52°C
- Increased co-transcriptional capping efficiency with cap analogs
- Decreased unwanted immunogenicity from RNA synthesized at higher temperature due to reduced dsRNA by-product formation

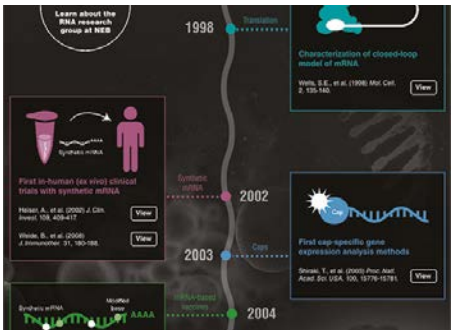
To learn more, please visit
www.neb.com/M0470

Ordering information:

Product	NEB #	Size
Hi-T7 RNA Polymerase (High Concentration)	M0470T	50.000 units
Hi-T7 RNA Polymerase	M0658S	5.000 units

Explore our interactive timeline of mRNA discoveries

Get caught up on key mRNA discoveries with our interactive timeline, featuring a selection of publications and resources from the last seven decades.



Discover these RNA Synthesis resources at **www.NEBrna.com**

Request from our website:

- RNA Technical Guide – Find in depth information on tools designed to streamline your RNA workflows
- RNA Synthesis Brochure – Learn more about NEB's products for RNA synthesis, which range from template generation to poly(A) tailing
- GMP-Grade* Reagents for RNA Synthesis Brochure – Learn about the benefits of GMP-grade materials available from NEB, and how they can be used in your mRNA synthesis workflow

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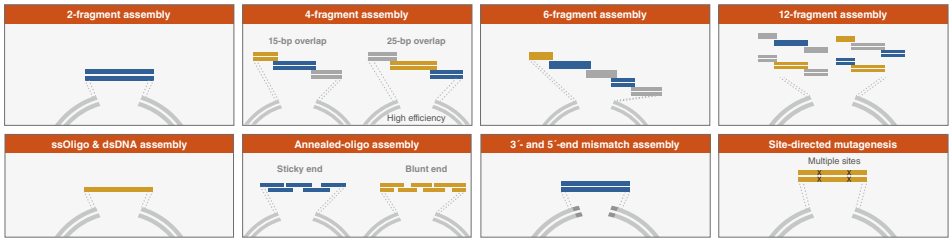

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Ordering information:

Product	NEB #	Size
NEBuilder HiFi DNA Assembly Master Mix	E2621S/ L/X	10 /50 / 250 rxns
NEBuilder HiFi DNA Assembly Cloning Kit	E5520S	10 rxns
NEBuilder HiFi DNA Assembly Bundle for Large Fragments	E2623S	20 rxns

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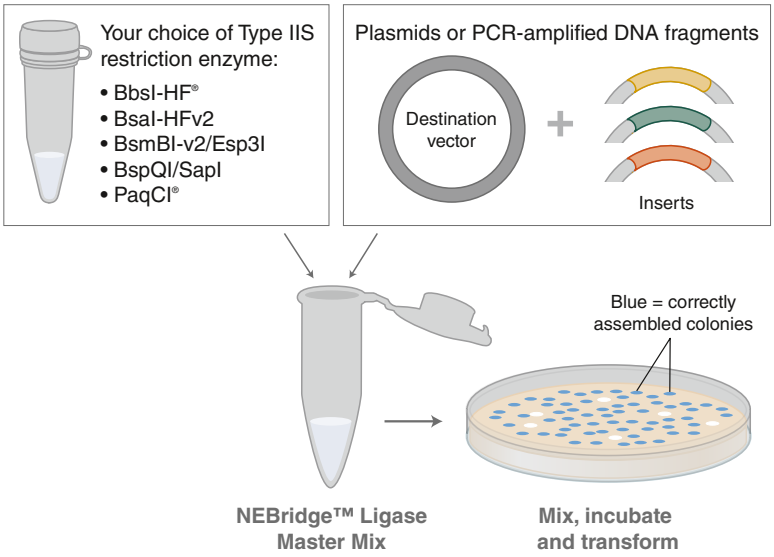
Offering flexibility and convenience for users, NEBridge Ligase Master Mix performs high efficiency and high-fidelity Golden Gate Assembly with a broad range of NEB Type IIS restriction enzymes.

NEBridge Ligase Master Mix is a 3X master mix for Golden Gate Assembly. Designed for use with NEB Type IIS restriction enzymes, this master mix contains T4 DNA Ligase in an optimized reaction buffer with a proprietary ligation enhancer. Users only need to choose their preferred NEB Type IIS restriction enzyme and add DNA substrates to be assembled. Low complexity single-fragment insertions, as well as moderate complexity (3–6 fragment) and high complexity (7–25+ fragment) assemblies, are all supported with this optimized reagent and accompanying protocols.



Advantages:

- Optimized for efficient and accurate Golden Gate Assembly
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- Ideal for ordered assembly of multiple fragments (2-25+) in a single reaction
- Can also be used for cloning single inserts and library construction
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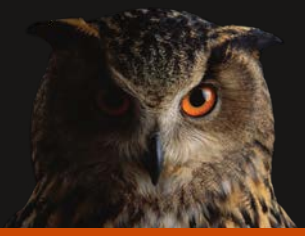
Ordering information:

Product	NEB #	Size
NEBridge Ligase Master Mix	M1100S	50 rxns



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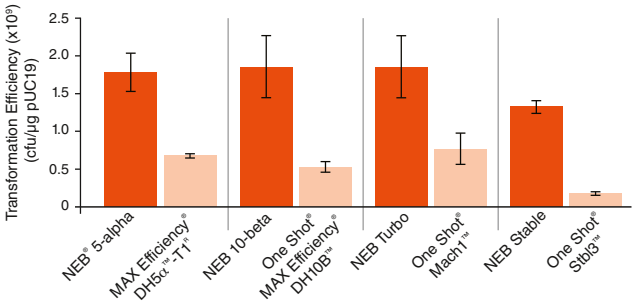
Benefit from high transformation efficiencies



Transformation efficiencies were compared using manufacturers' recommended protocols. Values shown are the average of triplicate experiments.

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FEATURES						
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Fast growth (< 8 hours)		•				
Toxic gene cloning			•			•
Large plasmid/BAC cloning				•		
Dam/Dcm-free plasmids					•	
Retroviral/lentiviral vector cloning						•
RecA-	•		•	•		•
FORMATS						
Chemically competent	•	•	•	•	•	•
Electrocompetent	•			•		
Subcloning	•					
96-well plate format	•			•		
384-well plate format	•					
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Advantages:

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- Compatible with NEBuilder HiFi DNA Assembly and NEBridge Golden Gate Assembly reactions, as well as ligation reactions. No dilution required!
- All strains are free of animal products and are T1 phage resistant
- Outgrowth medium and control plasmid are included
- Choose from a variety of convenient formats, including single-use tubes
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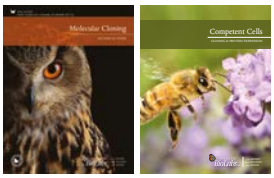

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LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG – lyophilized to be stored & shipped at room temperature

With the point-of-care market becoming more focused on the development of robust, accurate and cost-effective diagnostic tests for use outside of traditional hospital and laboratory settings, there is a growing need for reagents that can withstand ambient shipping and storage. Lyophilization is the preferred solution and is a well-established technology across a number of industries.

Bringing together expertise in enzyme development, manufacturing and lyophilization, NEB and Fluorogenics Limited have created shelf-stable, lyophilized products that do not sacrifice the high-performance qualities of their liquid counterparts. The first of these products includes a mixture of enzymes and inhibitors to enable robust detection of RNA via hydrolysis-probe-based RT-qPCR.

LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG (NEB #L4001) contains the same versatile features and strong performance as the liquid version: Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019). Performance in multiplexing applications has been optimized, with sensitive, linear detection of up to 5 targets across a range of inputs. The stable cake can be resuspended to make a 2X or 4X mix to accommodate a variety of sample input volumes.

Supplied as a lyophilized cake, the LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG enables sensitive detection of target RNA sequences in a room temperature-stable format.

Rehydration is rapid, and the lyophilized cake will typically dissolve within 1-2 seconds after addition of water. Note that air from the cake will naturally degas from solution over ~20 seconds or after gentle vortexing.

Advantages:

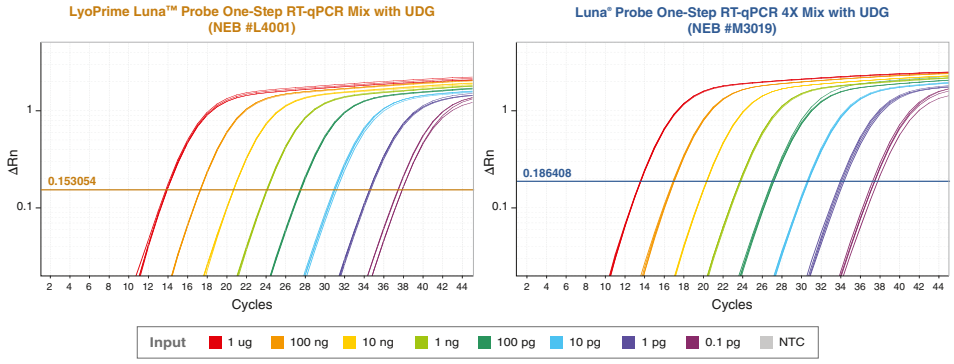
- Simply add nuclease-free water for rapid rehydration
- Store at room temperature for up to 2 years prior to rehydration
- Eliminate cold chain shipping requirements
- Same product performance as liquid format (#M3019)
- Developed in collaboration with Fluorogenics Limited, a wholly owned subsidiary of New England Biolabs, Inc.

To learn more, please visit
www.neb.com/L4001

Ordering information:

Product	NEB #	Size
LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG	L4001S	120 rxns
Nuclease-free Water	B1500S/L	25/100 ml

Lyophilized and liquid Luna RT-qPCR mixes demonstrate equivalent strong performance



RT-qPCR targeting human ß-actin was performed using either the LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG (NEB #L4001) or Luna Probe One-Step 4X Mix with UDG (NEB #M3019) over an 8-log range of input template concentrations (1 µg – 0.1 pg Jurkat total RNA) with 4 replicates at each concentration, run on an ABI QuantStudio 6 Flex real-time instrument. Reactions (20 µl) included primers at 400 nM each and and probe at 200 nM, and followed the product recommended cycling conditions.

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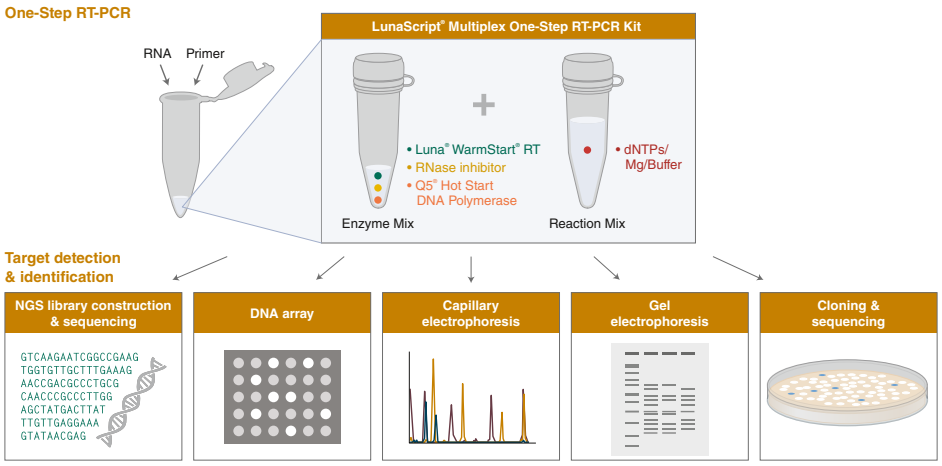
LunaScript® Multiplex One-Step RT-PCR Kit – for virtually "error-free" multiplexing PCR

The LunaScript Multiplex One-Step RT-PCR Kit offers a streamlined protocol for cDNA synthesis and extremely high fidelity PCR amplification in a single reaction. The 5X reaction mix contains dNTPs and is optimized for robust multiple target detection in a simple workflow. The 25X enzyme mix features Luna WarmStart Reverse Transcriptase and Q5 Hot Start High-Fidelity DNA Polymerase offering the highest fidelity amplification available making it an ideal choice for next-generation sequencing, library construction DNA arrays, fragment analysis, electrophoresis and traditional cloning/sequencing workflows.



The LunaScript Multiplex One-Step RT-PCR Kit requires only a RNA template and gene-specific primers to enable multiplex cDNA target synthesis and amplification in a single reaction. Amplified cDNA products can be detected or identified by downstream applications including next-generation sequencing, DNA arrays, fragment analysis, electrophoresis and traditional cloning/sequencing workflows.

Advantages:

- Closed-tube system with cDNA synthesis and endpoint PCR amplification in a single protocol
- Detect as low as 0.01 pg of human total RNA
- Highest fidelity multiplexing capacity supports use in ARTIC workflows, DNA arrays, cloning & sequencing etc.
- Aptamer-based enzyme control for room temperature setup and stability up to 24 hours

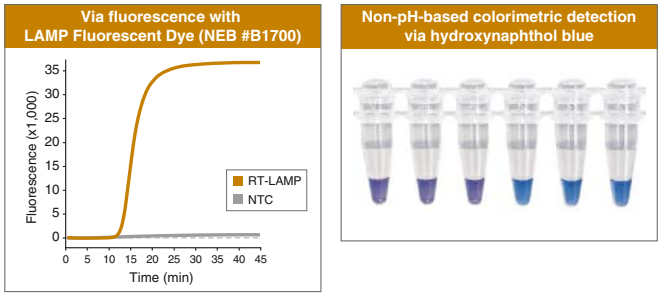
Ordering information:

Product	NEB #	Size
LunaScript Multiplex One-Step RT-PCR Kit	E1555S/L	50/250 rxns

To learn more, please visit
www.neb.com/E1555

WarmStart® Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)

The WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) is fully buffered and compatible with different sample types, enabling multiple detection methods including turbidity detection, real-time fluorescence detection, and end-point visualization such as colorimetric detection via a metal indicator (e.g., hydroxynaphthol blue). For real-time fluorescence detection, the master mix is available as a kit that includes 50X LAMP Fluorescent Dye. The inclusion of dUTP and thermolabile UDG enables carryover contamination prevention.



The WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) is compatible with multiple detection methods.

Advantages:

- Reduce the risk of carryover contamination with thermolabile UDG and dUTP included in the mix
- Set up reactions at room temperature with our unique dual WarmStart formulation
- Optimized performance for real-time fluorescence and endpoint visualization detection methods

Ordering information:

Product	NEB #	Size
WarmStart Multi-Purpose LAMP/ RT-LAMP 2X Master Mix with UDG	M1708S/L	100/500 rxns

To learn more, please visit
www.neb.com/M1708

The core of your NGS Library Prep

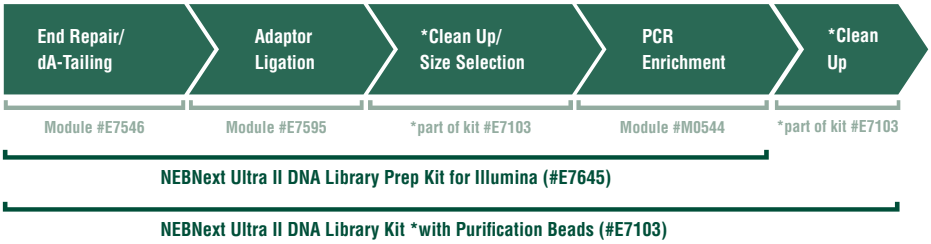
NEBNext® Ultra™ II reagents & kits: One central workflow for a wide range of applications – now featuring PCR-free workflows

As sequencing technologies continue to improve and applications expand, the need for compatibility with ever-increasing input amounts and sub-optimal sample quality grows. Reliability and high performance are critical, along with faster turnaround, higher throughput, and automation compatibility.

NEBNext's line of Ultra II DNA library prep reagents has expanded to include PCR-free workflows, powering high performance without the need for amplification. By eliminating the risk of PCR bias, libraries are a clearer reflection of biology.

The NEBNext Ultra II workflow lies at the heart of NEB's portfolio for next generation sequencing library preparation, with kits and modules for optimal flexibility. You can be assured your DNA libraries will be of the highest quality and yield, even when starting from extremely low input amounts.

The ULTRA II DNA WORKFLOW is available in convenient kit formats or modules:



Your benefits:

- Highest library yields and quality
- Fewer PCR-cycles /PCR-free optional
- Low input amounts

- „Hands on" time : <15 minutes
- Total time: Just ~2:30 – 3:00 hrs

Choose the convenient NEBNext Ultra II DNA Library Prep Kit for:

- Whole Genome Seq
- Standard & Low Input Seq
- ChIP-seq, NICE-seq, Cut&Run-Seq
- Exome Capture
- Targeted Sequencing
- FFPE-Material
- cfDNA ...

Advantages:

- Learn one central workflow and apply it to a whole suite of different applications
- Save time with streamlined, modular workflows, reduced hands-on time, and automation compatibility
- Benefit from low input amount requirements, fewer PCR cycyles and extremely uniform GC-coverage in all applications
- **NEW:** Now also available as PCR-free workflow to eliminate the risk of PCR bias

TOOLS & RESOURCES

- View performance data in our Technical Notes, which can be downloaded at **NEBNextUltraII.com**
- View the NEBNext Ultra II DNA protocol video for protocol steps, and tips for optimization
- Find hundreds of peer reviewed publications citing use of NEBNext Ultra II DNA on the product pages at **NEB.com**

The ULTRA II DNA WORKFLOW IS ALSO THE CORE OF:

Enzymatic Methyl-Seq (bisulfite-free)	Directional & non-directional RNA-seq	Enzymatic DNA Fragmentation System	Single Cell/ Low Input RNA Library Prep	SARS-CoV-2/ ARTIC Surveillance Sequencing

"NEB is currently the only company, which is offering really "automation friendly" library preparation kits. The volumes of components are calculated to cover for unavoidable deadvolumes, reactant volumes are in range of most automated platforms. The stability of the NEBNext chemistry allows a broad range of automation strategies."

– Dr. Jürgen Zimmermann,
Senior Engineer - Automation
GeneCore EMBL Heidelberg, Germany

Use the SELECTION CHART below to determine which NEBNext Ultra II products best suit your needs:

FEATURES	ULTRA II DNA LIBRARY PREP	ULTRA II FS DNA LIBRARY PREP
	Unprecedented performance, enabling lower inputs and fewer PCR cycles	Perform fragmentation, end repair, and dA-tailing with a single enzyme mix
ULTRA II INPUT AMOUNTS	500 pg – 1 µg of sheared DNA	100 pg – 500 ng of intact DNA
AVAILABLE WITH OR WITHOUT BEADS?	• With beads: NEB #E7103 • Without beads: NEB #E7645	• With beads: NEB #E7435 • Without beads: NEB #E7805
MODULES AVAILABLE?	• Ultra II End Repair/dA-tailing Module (NEB #E7546) • Ultra II Ligation Module (NEB #E7595)	• Ultra II FS DNA Module (NEB #E7810)
ULTRA II PCR-FREE INPUT AMOUNTS	250 ng – 1,000 ng of sheared DNA	50 ng – 500 ng of intact DNA
Available with or without beads?	• With beads: NEB #E7415 • Without beads: NEB #E7410	• With beads: NEB #E7435 • Without beads: NEB #E7430
Modules available?	• Ultra II End Repair/dA-tailing Module (NEB #E7546) • Ultra II Ligation Module (NEB #E7595)	• Ultra II FS DNA Module (NEB #E7810)
COMPATIBLE WITH FFPE DNA?	Yes – for improved performance, consider the NEBNext FFPE DNA Repair Mix (NEB #M6630)	Yes – for improved performance, consider the NEBNext FFPE DNA Repair Mix (NEB #M6630)
COMPATIBLE WITH METHYLOME ANALYSIS?	Yes – Methylome analysis is supported; however, we recommend NEBNext EM-seq™ (NEB #E7120)	No – not compatible due to potential for loss of methyl marks
COMPATIBLE WITH OXFORD NANOPORE TECHNOLOGIES?	Yes. We recommend the NEBNext Companion Module for Oxford Nanopore Technologies® Ligation Sequencing (NEB #E7180).	

Note: NEBNext Multiplex Oligos are available separately. Please visit **www.neb.com/oligos** for options.

Ordering Information:

Product	NEB #	SIZE
NEBNext Ultra II DNA Library Prep Kit for Illumina	E7645S/L	24/96 reactions
NEBNext Ultra II DNA Library Prep with Sample Purification Beads	E7103S/L	24/96 reactions
NEBNext Ultra II FS DNA Library Prep Kit for Illumina	E7805S/L	24/96 reactions
NEBNext Ultra II FS DNA PCR-free Library Prep with Sample Purification Beads	E7435S/L	24/96 reactions
NEBNext Ultra II End Repair/dA-Tailing Module	E7546S/L	24/96 reactions
NEBNext Ultra II Ligation Module	E7595S/L	24/96 reactions
NEW: NEBNext Ultra II DNA PCR-free Library Prep Kit for Illumina	E7410S/L	24/96 reactions
NEW: NEBNext Ultra II DNA PCR-free Library Prep with Sample Purification Beads	E7415S/L	24/96 reactions
NEW: NEBNext Ultra II FS DNA PCR-free Library Prep Kit for Illumina	E7430S/L	24/96 reactions
NEW: NEBNext Ultra II FS DNA PCR-free Library Prep w. Sample Purification Beads	E7435S/L	24/96 reactions
NEBNext Ultra II FS DNA Module	E7810S/L	24/96 reactions
NEBNext FFPE DNA Repair Mix	M6630S/L	24/96 reactions
NEBNext Enzymatic Methyl-seq Kit	E7120S/L	24/96 reactions
NEBNext Enzymatic Methyl-seq Conversion Module	E7125S/L	24/96 reactions
NEBNext Companion Module for Oxford Nanopore Technologies® Ligation Sequencing	E7180S	24 reactions

Free Sample

Limited Offer: Please visit
www.neb.com/specialoffers

For more information incl. an overview of all NEBNext Ultra II Kits and modules etc., please visit **www.NEBNext.com**

NEB Restriction Enzyme Buffers and Formulations with Recombinant Albumin (rAlbumin)

NEB understands that there is an increased need to move away from animal-containing products such as Bovine Serum Albumin (BSA) while maintaining comparable performance. We are excited to announce that NEB has begun transitioning our restriction enzyme formulations and buffers to contain rAlbumin instead of BSA.

The buffer switch was completed in early 2022. Enzyme formulations will take a little longer and may take several years before all are switched. You can continue to use BSA- or rAlbumin-containing buffers or formulations.

We feel that moving away from animal-containing products is a step in the right direction and can offer this enhancement at the same price.

Enzyme Formulation Changes:

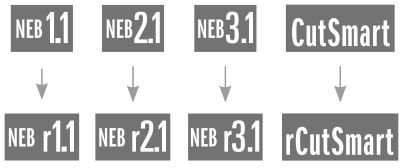
- Use of rAlbumin in enzyme formulations enables them to be used in situations where BSA-free reagents are required
- The catalog number will not change when a restriction enzyme is reformatted with rAlbumin
- There is no difference in enzyme performance when using an enzyme formulated with BSA versus rAlbumin, either can be used in your reaction
- Certificates of Analysis, Certificates of Origin and Specifications will be updated to reflect the change

Find out which enzyme formulations contain rAlbumin by visiting the product page of your enzyme of interest. A message will be included at the top of the product webpage of your enzyme of interest and in the "Specifications and Change Notification" tab - the message will indicate the starting lot number that contains rAlbumin. Please note that all higher lot numbers will also contain rAlbumin.

Learn more about restriction enzyme formulation changes by visiting
www.neb.com/BSA-free2

Buffer Changes:

NEBuffer names changed to include a lowercase "r" in front of the buffer. You can easily tell which version you have by looking at the name on the buffer vial.



Learn more about buffer changes by visiting
www.neb.com/BSA-free

Any questions regarding the switch to rAlbumin? Please contact
techsupport.fr@neb.com

France
New England Biolabs France SAS
Genopole Campus 1, Bâtiment 6
5 rue Henri Desbruères
91030 EVRY cedex
Toll Free Telephone: 0800 100 632
Toll Free FAX : 0800 100 610
info.fr@neb.com

www.neb-online.fr

USA (Headquarter)
New England Biolabs, Inc.
Telephone: (978) 927-5054
Toll Free: (U.S. Orders) 1-800-632-5227
Toll Free: (U.S. Tech) 1-800-632-7799
Fax: (978) 921-1350
info@neb.com

United Kingdom
New England Biolabs (UK), Ltd.
Call Free 0800 318486
info.uk@neb.com

Germany & Austria
New England Biolabs GmbH
Free Call 0800/246 5227 (Germany)
Free Call 00800/246 52277 (Austria)
info.de@neb.com

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